

PATENTIN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant: Grabowski and Du : Paper No:
Serial No. 09/775,517 : Group Art Unit: 1651
Filed: February 2, 2001 : Examiner: Jon Weber
For: LIPID HYDROLYSIS THERAPY FOR ATHEROSCLEROSIS AND RELATED DISEASES

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

David Hui Ph.D., declares that:

- Convinced
27 May 2004
JH*
- 1) I am a Professor of Pathology & Laboratory Medicine at the University of Cincinnati. I received a Ph.D. in Biochemistry from Indiana University in 1979. I have worked at the University of Cincinnati since 1987. My areas of expertise include utilization of cellular, molecular, and genetic approaches to explore the structure and function of lipid transport proteins, as well as their roles in normal physiology and pathophysiologic conditions such as coronary artery disease, diabetes and obesity, hyperlipidemia, and nutrient digestion and transport.
 - 2) I am familiar with the Grabowski and Du lysosomal acid lipase (LAL) therapy patent application, which describes a method to diminish and/or eliminate atherosclerotic plaques, in mammals, through direct and indirect treatment of these plaques, *in situ*, using proteins and/or polypeptides that are capable of lipid removal, primarily through hydrolysis. The lipid hydrolyzing protein or polypeptide targets receptors in and/or on the cell which lead to uptake into the

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lysosome. Compositions used for practicing this invention include lipid hydrolyzing proteins or polypeptides, and in particular, lysosomal acid lipase (LAL).

- 3) A hallmark of atherosclerosis is the deposition of cholesterol and cholesterol-rich macrophage foam cells in the arterial wall. Increased circulating levels of low density lipoproteins (LDL) as well as decreased level of high density lipoproteins (HDL) are major risk factors for premature atherosclerosis. The mechanism by which increased LDL level contributes to atherosclerosis is well established. The prolonged circulation of LDL results in their increased susceptibility to oxidative modification and the oxidized LDL can be internalized into macrophages by a non-regulated scavenger receptor mediated endocytotic pathway. The internalized oxLDL are delivered to the lysosomes where the cholesteryl esters associated with the lipoproteins are hydrolyzed by lysosomal acid lipase (LAL), the unesterified cholesterol liberated from this hydrolysis exits the lysosomes where it can be esterified into cholesteryl esters by acyl-CoA:cholesterol acyltransferase (ACAT). The cholesteryl esters are stored in the cytoplasm as lipid droplets and the accumulation of these droplets result in foam cell formation.

- 4) I have read and am familiar with the Grabowski and Du patent application. In the Grabowski and Du patent application, LAL is used as the enzyme to promote cholesteryl ester hydrolysis. Note that LAL and HSL have distinct intracellular location and presumed to have distinct functions in intracellular cholesterol homeostasis. Whereas HSL is a cytosolic enzyme, thus capable of participating in hydrolyzing the cytoplasmic cholesteryl ester droplets in macrophage to limit foam cell formation, LAL is a lysosomal enzyme that participates in liberating

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unesterified cholesterol from cholestryl esters entering the cells through endocytosis.

- 5) In my scientific opinion, I believe that the exogenous LAL therapy as described by Grabowski and Du, in all likelihood should not work to clear atheroslerotic plaque, and if it did work as stated, it would have limited utility. Furthermore, if this LAL therapy is shown to successfully clear atherosclerotic plaque, such results would be wholly unexpected and surprising given the current state of research in this field.
- 6) I hereby declare that all statements made herein of my own knowledge are true and that all statements made on the information and belief are believed to be true; and further that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued therein.

David T. Hui

David Hui, Ph.D.

3/26/04

The Role of LAL

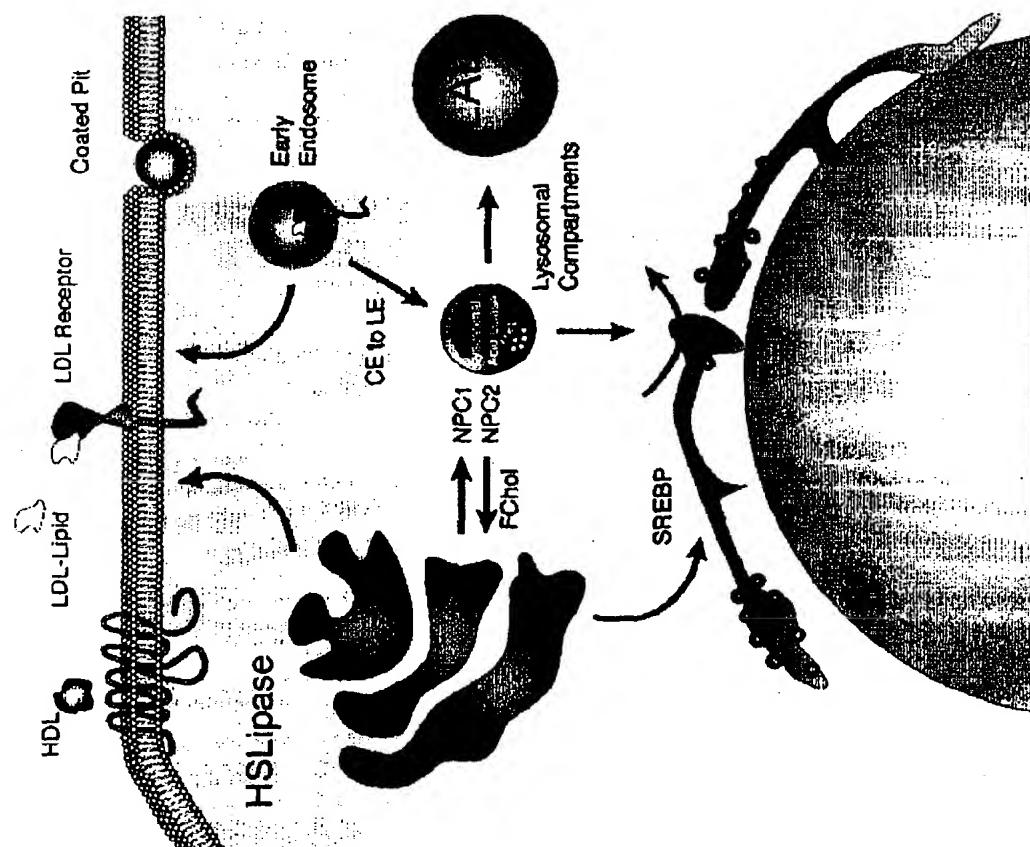
Common pathway to control of cholesterol
and triglyceride metabolism

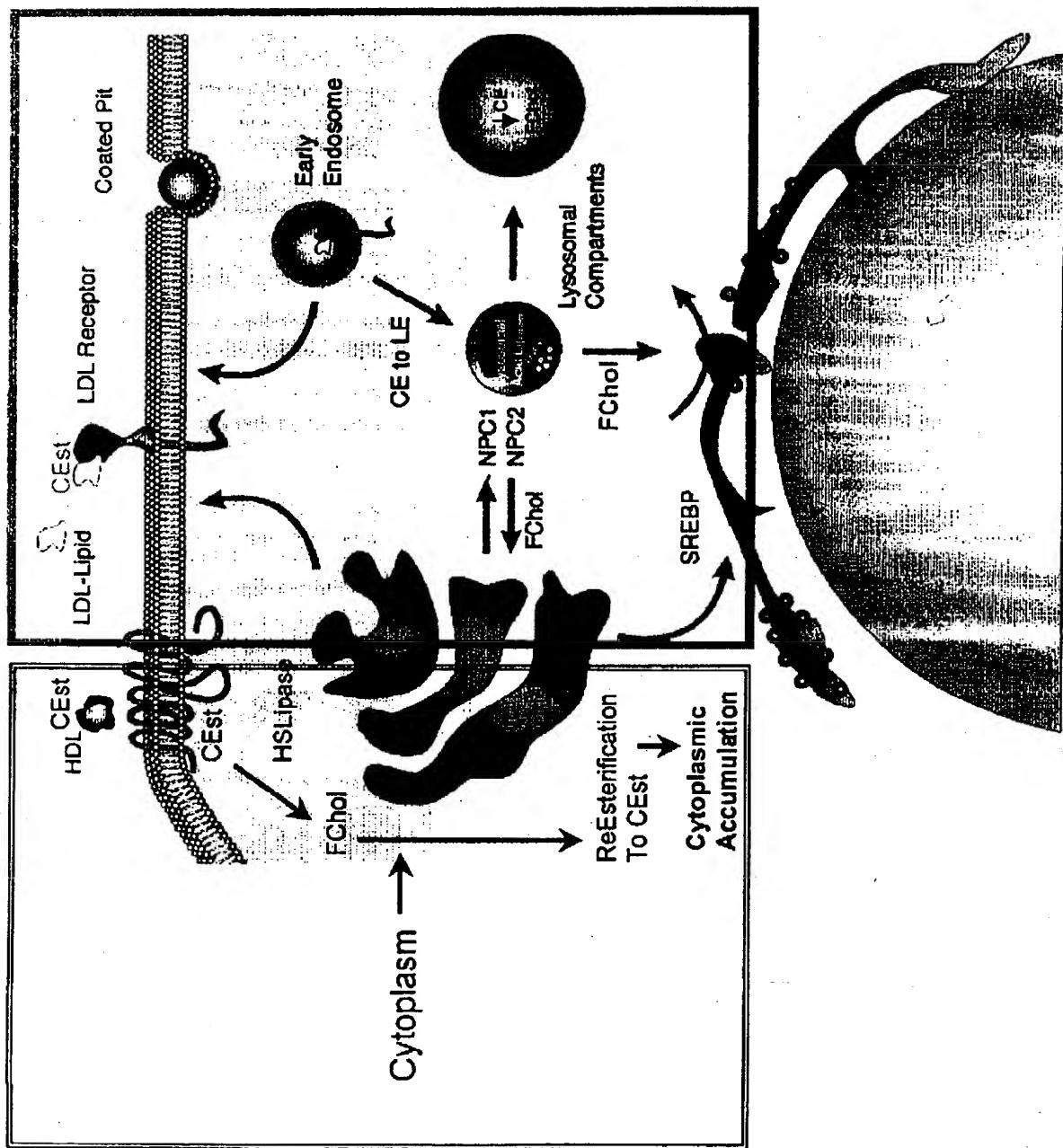
The Metabolic Role of LAL

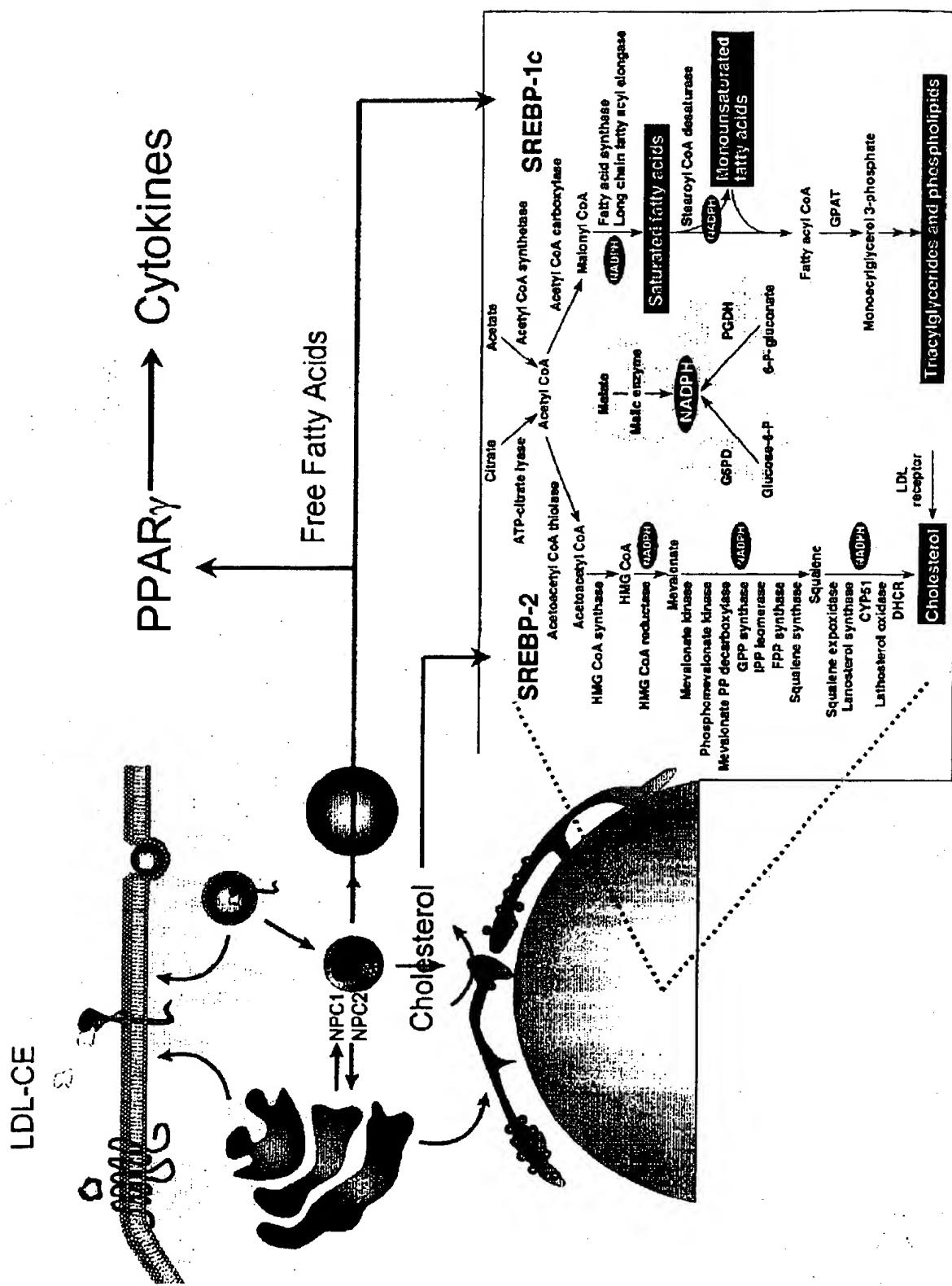
Hydrolysis of cholestryl esters and triglycerides delivered to Lysosome by the LDL receptor-mediated endocytotic pathway
Liberated free cholesterol and fatty acids signal many downstream events

The Metabolic Role of Hormone Sensitive Lipase (HSLipase)

Hydrolysis of cholestryl esters delivered to
the Cytoplasm by HDL mediated transfer
Liberated free cholesterol is re-esterified

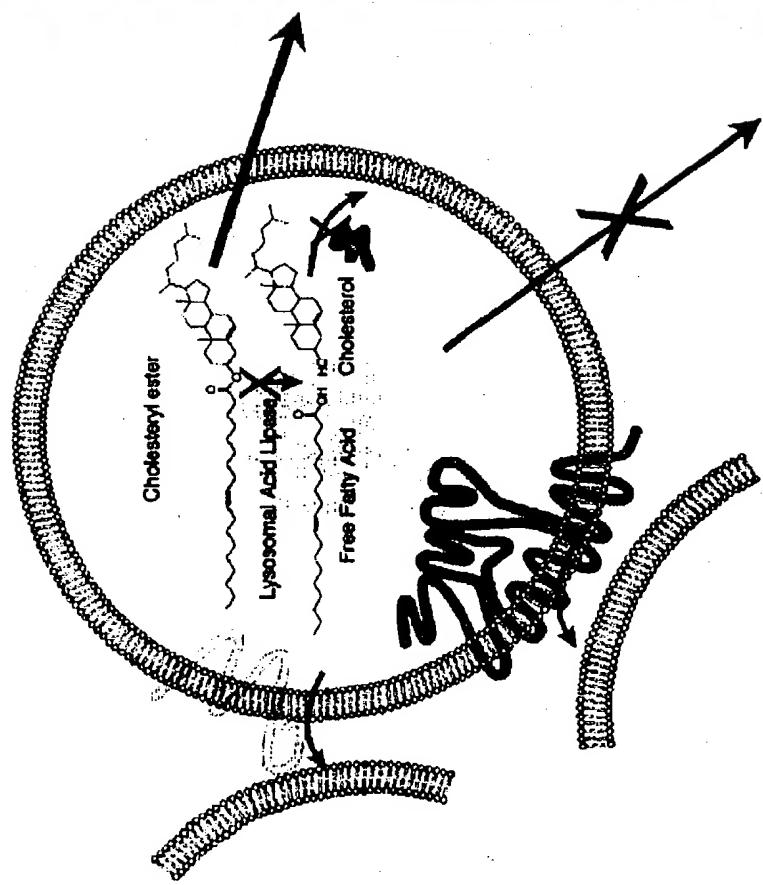








lysosomal Acid Lipase Deficiency



LAL and HSLipase Are Different Enzymes

- Different cell compartments
 - LAL in lysosomes
 - HSLipase in cytoplasm
- Different amino acid sequences
- Different genes
- Different post-translational modifications
- Different developmental/tissue expression
- Different functions in cell

Amino Acid Alignment (hLAL and h-HSL)

<i>hLAL</i> 39	[K M F L G L Y C L W P - - - L H E S Y - - -	397	<i>h-HSL</i> 39	[D L T M T Q S V T E D N I A F S G G E T A	397
<i>hLAL</i> 39	- -	407	<i>hLAL</i> 39	[Q R L S G V F A G V R E Q A L G L E P A L G R L L G V A . L	407
<i>hLAL</i> 39	T A [N M S E I S Y W F P S E E Y E T E	407	<i>h-HSL</i> 39	F D [P A Y R S V H T R C C L A H H K S L .	407
<i>hLAL</i> 39	D [C L N I P H G K N S D K G - - - - -	417	<i>hLAL</i> 39	R - S N R S I F F T S N L A E L E A Y L A A L T	417
<i>hLAL</i> 39	R - G Y . R R H . P G V . F G D . G L	417	<i>h-HSL</i> 39	Q I R A L V Y Y A Q R L L V T . P G V . F G D . G L	417
<i>hLAL</i> 39	S S N W V T N A N - - - - - S S I L A D F D	427	<i>h-HSL</i> 39	F I R E Y Y T H K R C Q F T P R P A .	427
<i>hLAL</i> 39	A D L G C F Y G L G F	427			

150
h-LAL 38 V W M G N R - - - - - N T S [REDACTED] S Q D - -
h-HSL 38 F L Q T I [REDACTED] E H K [REDACTED] K N E T G [REDACTED] A A S [REDACTED] S L

150
h-LAL 38 [REDACTED] 230
h-HSL 38 [REDACTED]

150
h-LAL 38 F T S G R F A I D P E I R G A E F F E R I T Q N L D V H F W K

150
h-LAL 38 230
h-HSL 38 [REDACTED]

150
h-LAL 38 E [REDACTED] F W N I T M E V L S S L A N M A S A T V R V S R L L S L

150
h-LAL 38 - - - S Y D E M [REDACTED] K Y D [REDACTED] P [REDACTED] N F I [REDACTED] N [REDACTED] S P P [REDACTED] A [REDACTED] P P E A F A L . I L . T G P G P

150
h-LAL 38 [REDACTED] 230
h-HSL 38 V L V R L I S S Y D L R E G Q D S E . . . S . G Q R

150
h-LAL 38 T I [REDACTED] I A F S [REDACTED] E L A K R [REDACTED] K M [REDACTED] P [REDACTED] S V A
h-HSL 38 L E [REDACTED] P R P Q [REDACTED] R S R S L [REDACTED] V H [REDACTED] F [REDACTED] Q T S Q . P I F . . . G V A

4407
H&L 39 FACTS [REDACTED] - M K L R
H-H&L 39 R S H E [REDACTED] - M K L P E P
P Y L K S W A Q E I L G A P I I S I D Y S A

4407
H&L 39 L D H I K D L [REDACTED] D K E F P Q S F K W L T [REDACTED]
H-H&L 39 F R A [REDACTED] E E C F [REDACTED] Y C W A [REDACTED] K H C [REDACTED] L G S T [REDACTED] E [REDACTED]
P L F
A L G . C

4407
H&L 39 [REDACTED] K E C [REDACTED] - - - - -
H-H&L 39 - - - D S G [REDACTED] - - - - -
T H G N L C F T V A L R A A A Y G V R V P D G

4407
H&L 39 - - - - -
H-H&L 39 [REDACTED] - - - - -
I M A A Y P A T M I Q P A A S P S R L L S L

4407
H&L 39 N M S R [REDACTED] D [REDACTED] T - - - T E P A G T S Q N L H W
H-H&L 39 L S K C [REDACTED] S [REDACTED] A [REDACTED] D [REDACTED] N S D Q K [REDACTED] L G M G L
V Y G A K T E H S . M

4407
H&L 39 S Q A V K F Q K F Q A D W S [REDACTED] - - - - -
H-H&L 39 V R R D T A L L R D [REDACTED] R I A [REDACTED] F G S S W L N S F L E L S G R K

550 - - - - - AKNYFHYN SYPPI
 550 - - - - - SEAAIAQPGPLG
 550 - - - - - Q T

550 - - - - - DMLPTVWSGHWLA
 550 - - - - - SLRNSTSSIT
 550 - - - - - G D F EMSLSA
 550 - - - - - K

660 - - - - - Y I T
 660 - - - - - S F P
 660 - - - - - DVNL PEDAGEEAEKNEL

660 - - - - - QITNFESEWE
 660 - - - - - MDRGVAAFGF
 660 - - - - - SP L PE H RSSQGATQMP

660 - - - - - IWGDWRLYN
 660 - - - - - MSPLDNSMLK
 660 - - - - - LYSSPIVKNPFLAP SLPPPVHI

770 - - - - - KINMMYQ
 770 - - - - - DSMALR NLGQPVTLRV

MAIL 33
A-MAIL 33

V E D I P H G F I T I A A L C R E T R Q A A E I C V E R I R

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